Form PTO-1390 (REV 10-2000) U S DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

ATTORNEY'S DOCKET NUMBER: 0623.0950000/EKS/GLL

U.S APPLICATION NO. (IF KNOWN, SEE 37 C.F.R. § 1.5): To be assigned 09/719055

INTERNATIONAL APPLICATION NO	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/GB99/01828	9 June 1999	9 June 1998

TITLE OF INVENTION: **Predictive Test For Pre-Eclampsia**

APPLICANT(S) FOR DO/EO/US: WALD, Nicholas John and REDMAN, Christopher

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☒ This is an express request to begin national examination procedures (35 U.S.C. 371(f)).
4. ☒ The US has been elected by the expiration of 19 months from the priority date (PCT Article 31).
5. ☒ A copy of the International Application as filed (35 U.S.C. 371(c)(2))
   a. ☐ is attached hereto (required only if not communicated by the International Bureau).
   b. ☒ has been communicated by the International Bureau.
   c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☐ An English language translation of the International Application as filed (35 U.S.C. 371(c)(2)).
7. ☒ Amendments to the claims of the International application under PCT Article 19 (35 U.S.C. 371(c)(3))
   a. ☐ are attached hereto (required only if not communicated by the International Bureau).
   b. ☐ have been communicated by the International Bureau.
   c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
   d. ☒ have not been made and will not be made.
8. ☐ An English language translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 372(c)(3)).
9. ☐ An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).
10. ☐ An English language translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11. to 16. below concern other document(s) or information included:

11. ☒ An Information Disclosure Statement under 37 C.F.R. 1.97 and 1.98.
12. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 C.F.R. 3.28 and 3.31 is included.
13. ☒ A FIRST preliminary amendment.
    ☐ A SECOND or SUBSEQUENT preliminary amendment.
14. ☐ A substitute specification.
15. ☐ A change of power of attorney and/or address letter.
16. ☒ Other items or information: 1) A copy of International Publication No. WO 99/64860; 2) Authorization To Treat A Reply As Incorporating An Extension Of Time Under 37 C.F.R. § 1.136(a)(3); 3) Form PTO-1449 citing (11) eleven documents; and 4) A copy of documents AA1, AL1, AM1, AN1, AR1, AS1, AT1, AR2, AS2, AT2, and AR3.

U.S. APPLICATION NO (if known, see 37 C F R 1 50) To be assigned 09/719055	INTERNATIONAL APPLICATION NO PCT/GB99/01828	ATTORNEY'S DOCKET NUMBER 0623.0950000/EKS/GLL

17. ☒ The following fees are submitted:	CALCULATIONS	PTO USE ONLY
Basic National Fee (37 CFR 1.492(a)(1)-(5)): Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO **$1000.00**		
International preliminary examination fee (37 CFR 1 482) not paid to USPTO but International Search Report prepared by the EPO or JPO **$860.00**		
International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1 445(a)(2)) paid to USPTO **$710.00**		
International preliminary examination fee paid to USPTO (37 CFR 1.482) but all claims did not satisfy provisions of PCT Article 33(1)-(4) **$690.00**		
International preliminary examination fee paid to USPTO (37 CFR 1 482) and all claims satisfied provisions of PCT Article 33(2)-(4) ..... **$ 100.00**		
ENTER APPROPRIATE BASIC FEE AMOUNT =	$860.00	
Surcharge of **$130.00** for furnishing the oath or declaration later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492(e)).		

Claims	Number Filed		Number Extra	Rate		
Total Claims	- 20	=		X **$18.00**	$0	
Independent Claims	- 3	=		X **$80.00**	$0	
Multiple dependent claim(s) (if applicable)				+ **$270.00**	$0	
TOTAL OF ABOVE CALCULATIONS				=	$0	
☐ Applicant claims small entity status See 37 CFR 1 27. The fees indicated above are reduced by 1/2.					$0	
SUBTOTAL				=	$860.00	
Processing fee of $130.00 for furnishing the English translation later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492(f)).				+	$0	
TOTAL NATIONAL FEE				=	$860.00	
Fee for recording the enclosed assignment (37 CFR 1 21(h)) The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). **$40.00** per property				+	$	
TOTAL FEES ENCLOSED				=	$860.00	
					Amount to be refunded:	$
					charged:	$

a. ☒ A check in the amount of $860.00 to cover the above fees is enclosed.

b. ☐ Please charge my Deposit Account No. _____ in the amount of $ _____ to cover the above fees. A duplicate copy of this sheet is enclosed.

c. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 19-0036. A duplicate copy of this sheet is enclosed.

NOTE: Where an appropriate time limit Under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO
STERNE, KESSLER, GOLDSTEIN & FOX P.L.L.C.
1100 New York Avenue, NW, Suite 600
Washington, D.C. 20005-3934

SIGNATURE

Eric K. Steffe
NAME

36,688
REGISTRATION NUMBER

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:	
WALD *et al.*	Art Unit: To be assigned
Appl. No. To be assigned (National Phase of International Appl. No. PCT/GB99/01828, filed June 9, 1999)	Examiner: To be assigned
Filed: Herewith	Atty. Docket: 0623.0950000/EKS/GLL
For: **Predictive Test for Pre-Eclampsia**	

Preliminary Amendment

Commissioner for Patents
Washington, D.C. 20231

Sir:

In advance of substantive examination in the above identified matter, please amend the application as follows:

In the Specification:

Please amend the specification as follows:

At page 1, after the title, please insert the following:

-- CROSS REFERENCE TO RELATED APPLICATIONS

The present application is the National Phase of International Application No. PCT/GB99/01828, filed June 9, 1999.--

At page 1, after the Cross Reference and before line 3 of the text, please insert

--BACKGROUND OF THE INVENTION

Field of the Invention--;

and before line 6 of the text, please insert --Related Art--.

At page 2, before line 20, please insert --BRIEF SUMMARY OF THE INVENTION--; and

after line 25, please insert --DETAILED DESCRIPTION OF THE INVENTION--.

At page 7, before line 2, please insert --BRIEF DESCRIPTION OF THE DRAWINGS--.

At page 8, before line 7, please insert --EXAMPLES--.

In the Claims:

At page 21 and before claim 1, please delete "CLAIMS" and insert therefor --WHAT IS CLAIMED IS:--.

Please cancel claims 2, 12 and 17 without prejudice to or disclaimer of the subject matter thereof.

Please rewrite the claims as follows:

1. (Once Amended) A method of predicting the risk of pre-eclampsia in a pregnant woman, the method comprising the steps of:

(a) obtaining a sample of blood from the woman;

(b) subsequently assaying the sample for the levels of free β-human chorionic gonadotrophin (free β-hCG) and Inhibin A present in the sample; and

(c) determining the risk of pre-eclampsia using the measure levels of free β-human chorionic gonadotrophin (free β-hCG), [and] Inhibin A, and unconjugated oestriol (uE_3) present in the sample.

3. (Once Amended) A method as claimed in claim 1 [or claim 2], in which the method is carried out after 20 weeks of pregnancy[,].

5. (Once Amended) A method as claimed in any of claims [1 to 4] 1, 3 or 4, in which the determination of risk in step (c)[,] is undertaken by comparing the levels of free β-human chorionic gonadotrophin (free β-hCG), [and] Inhibin A and unconjugated oestriol (uE_3) present in the sample with those in a control sample.

8. (Once Amended) A method as claimed in claim 7, in which the estimation of risk consists of multiplying the likelihood ration by the background risk for pre-eclampsia.

9. (Once Amended) A method as claimed in any one of claims [1 to 8] 1 or 3 to 8, the method further comprising a step (d) of re-expressing each measured screening marker level as a multiple of the median level of the respective screening marker in unaffected pregnancies of the same gestational age as the fetus of the pregnant woman.

11. (Once Amended) An apparatus for determining whether a pregnant woman is at an increased risk of pre-eclampsia, the apparatus comprising:

(a) data input means for inputting a measurement of the serum levels of Inhibin A_2 [and] free β-human chorionic gonadotrophin (free β-hCG) and unconjugated oestriol (uE_3) in a sample obtained from said pregnant woman; and

(b) calculation means for determining the risk of pre-eclampsia using the input levels of the serum markers Inhibin A_2 [and] free β-human chorionic gonadotrophin (free β-hCG) and unconjugated oestriol (uE_3).

13. (Once Amended) An apparatus as claimed in claim 11 [or claim 12], in which the calculation means is arranged to determine the risk of pre-eclampsia by deriving the likelihood ratio for pre-eclampsia using a multivariate analysis based on distribution parameters derived from a set of reference data.

15. (Once Amended) An apparatus as claimed in any one of claims [11 to 14] 11, 13 or 14, in which the apparatus further comprises (c) means for re-expressing the levels of each input screening marker as a multiple of the median level of the respective screening marker in unaffected pregnancies of the same gestational age as the fetus of the pregnant [women] woman and supplying the re-expressed screening marker levels to said calculation means.

16. (Once Amended) A kit for predicting the onset of pre-eclampsia in a pregnant woman, comprising means for assaying a sample from the [women] <u>woman</u> for the levels of free β-human chorionic gonadotrophin (free β-hCG), [and] Inhibin A <u>and unconjugated oestriol (uE_3)</u> present in the sample.

Remarks

By the foregoing amendments, Applicants have amended the international application to place the specification and claims into proper format for U.S. practice. The amendments to claims 1, 3, 5, 8, 9, 11, 13, 15 and 16 have been made to amend claim dependencies and to amend obvious typographical errors. Support for amended claims 1, 5, 11 and 16 can be found in the specification, *inter alia*, at page 3, lines 23-26; and in original claims 2, 12 and 17. Hence, no new matter has been added by the foregoing amendments, and entry and consideration of the same are respectfully requested.

Conclusion

It is not believed that extensions of time or fees for net addition of claims are required beyond those that may otherwise be provided for in documents accompanying this paper. However, if additional extensions of time are necessary to prevent abandonment of this application, then such extensions of time are hereby petitioned under 37 C.F.R. § 1.136(a), and any fees required therefor (including fees for net addition of claims) are hereby authorized to be charged to our Deposit Account No. 19-0036.

It is respectfully believed that this application is now in condition for examination. Early notice to this effect is earnestly solicited.

If the Examiner believes, for any reason, that personal communication will expedite prosecution of this application, the Examiner is invited to telephone the undersigned at the number provided.

Respectfully submitted,

STERNE, KESSLER, GOLDSTEIN & FOX P.L.L.C.

Eric K. Steffe
Attorney for Applicants
Registration No. 36,688

Date: December 7, 2000
1100 New York Avenue, N.W.
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prelim. amendm. rev

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:	
WALD *et al.*	Art Unit: To be assigned
Appl. No. 09/719,055 U.S. National Phase of Int'l. Appl. No.: PCT/GB99/01828; Filed: June 9, 1999	Examiner: To be assigned
For: **Predictive Test for Pre-Eclampsia**	Atty. Docket: 0623.0950000/EKS/GLL

Second Preliminary Amendment

Commissioner for Patents
Washington, D.C. 20231

Sir:

In advance of substantive examination in the above identified matter, please amend the application. This Amendment is provided in the following format:

(A) A clean version of each replacement paragraph/section/claim along with clear instructions for entry;

(B) Starting on a separate page, appropriate remarks. MPEP 714; and

(C) Starting on a separate page, a marked-up version entitled: "Version with markings to show changes made."

Amendments

Please substitute the 1st full paragraph after the title on page 1 (which was amended in a preliminary amendment filed December 7, 2000), with the following paragraph:

CROSS REFERENCE TO RELATED APPLICATIONS

The present application is the National Phase of International Application No. PCT/GB99/01828, filed June 9, 1999, which was published in English.

Remarks

By the foregoing amendment, Applicants have amended the international application to place the specification into proper format for U.S. practice. In particular, Applicants have amended the specification to indicate that the international application was published in English. Hence, no new matter has been added by the foregoing amendment, and entry and consideration of the same is respectfully requested.

Conclusion

It is not believed that extensions of time or fees for net addition of claims are required beyond those that may otherwise be provided for in documents accompanying this paper. However, if additional extensions of time are necessary to prevent abandonment of this application, then such extensions of time are hereby petitioned under 37 C.F.R. § 1.136(a), and any fees required therefor (including fees for net addition of claims) are hereby authorized to be charged to our Deposit Account No. 19-0036.

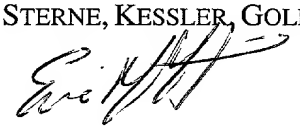
It is respectfully believed that this application is now in condition for examination. Early notice to this effect is earnestly solicited.

If the Examiner believes, for any reason, that personal communication will expedite

prosecution of this application, the Examiner is invited to telephone the undersigned at the number provided.

Respectfully submitted,

STERNE, KESSLER, GOLDSTEIN & FOX P.L.L.C.



Eric K. Steffe
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Date: July 2, 2001

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2nd prelim. amendm

Version with markings to show changes made

First full paragraph after the title on page 1:

CROSS REFERENCE TO RELATED APPLICATIONS

The present application is the National Phase of International Application No. PCT/GB99/01828, filed June 9, 1999, which was published in English.

PREDICTIVE TEST FOR PRE-ECLAMPSIA

The present invention relates to a test which can be used to predict pre-eclampsia in pregnant women.

Pre-eclampsia is a disorder of human pregnancy which affects around 5 to 10% of pregnancies. The underlying cause of pre-eclampsia remains unclear in spite of extensive clinical and basic research. Pre-eclampsia is the definition given to the condition in pregnancy in which elevated blood pressure is associated with proteinuria. Pre-eclampsia is distinct from eclampsia which is additionally associated with convulsions. Pre-eclampsia is defined in Souhami & Moxham *Textbook of Medicine*, Second edition, Churchill Livingstone (1994), as an abnormal rise in blood pressure between the first and second halves of pregnancy of ≥ 30/20 mmHg, with abnormal urate levels of >0.35 mmol/l at 32 weeks or >0.4mmol/l thereafter, associated with proteinuria, impaired renal function and clotting disorders. The consequences of pre-eclampsia are serious and include reduced uteroplacental perfusion, foetal growth retardation, pre-term birth, and increased foetal and maternal morbidity and mortality.

There have been many attempts to provide a reliable predictive test for pre-eclampsia. Previous suggestions have involved assays for the levels of circulating biochemical markers in the mother's blood but to date the scientific literature on this issue is contradictory and inconclusive. The following hormones have all been identified as possible markers in an elevation of levels might be predictive of pre-eclampsia in maternal plasma: progesterone, oestradiol, total human chorionic gonadotrophin (hCG), corticotrophin-releasing factor (CRF), adrenocorticotrophin (Muller *et al Am. J. Obst. Gynecol.* **175** 37-40 (1996); Ashour *et al Am. J. Obst. Gynecol.* **176** 438-444 (1997); Hsu *et al Am. J. Obst. Gynecol.* **170** 1135-1138 (1994); Wenstrom *et al A. J. Obst. Gynecol.* **171** 1038-1041 (1994)). Conversely, levels of oestriol, human placental lactogen and cortisol are unchanged or decreased. Whilst circulating CRF has been proposed as a prognostic marker for pre-eclampsia, treatment of hypertension does not

influence maternal CRF levels and nor has any correlation been found between CRF levels and mean blood pressure.

Other possible markers which have been suggested are Activin A and Inhibin A. Activin is a hypophysiotrophic factor produced by the placenta which is know to act as a growth factor having activity in modulating cell growth and differentiation. Currently, there are three forms of activin which are recognised to exist as homodimeric proteins: Activin A ($\beta_A\beta_A$), Activin AB ($\beta_A\beta_B$) and Activin B ($\beta_B\beta_B$) in which the subunits are linked by disulphide bridges. Inhibins are heterodimeric proteins consisting of $\alpha\beta_A$ (Inhibin A) and $\alpha\beta_B$ (Inhibin B) subunits also linked by disulphide bridges. Additionally monomeric Inhibin α subunits are present in the circulation and follicular fluid. Inhibin is thought to have an endocrine role which inhibits pituitary production of follicle-stimulating hormone (FSH). Muttikrishna *et al* (*The Lancet* **349** 1285-1288 (1997)) have proposed that Activin A and Inhibin A might be suitable markers for the onset of pre-eclampsia. These proteins were suggested because they were thought to be more sensitive markers than hCG or corticotrophin-releasing hormone where there is a considerable overlap in elevated hormone levels between control and pre-eclamptic women.

However, it has now been found that a predictive test for pre-eclampsia which is based on levels of free β-human chorionic gonadotrophin (free β-hCG) and Inhibin A can in fact provide a surprisingly improved level of predictiveness over previously known tests. The present invention describes a system of screening for pre-eclampsia, in which a single risk estimate is derived from measurements carried out on biochemical samples obtained during pregnancy.

According to a first aspect of the invention there is provided a method of predicting the risk of pre-eclampsia in a pregnant woman, the method comprising the steps of:

(a) obtaining a sample of blood from the woman;

(b) subsequently assaying the sample for the levels of free β-human chorionic gonadotrophin (free β-hCG) and Inhibin A present in the sample; and

(c) determining the risk of pre-eclampsia using the measured levels of free β-human chorionic gonadotrophin (free β-hCG) and Inhibin A present in the sample.

Methods according to the present invention are carried out *ex vivo*. In the step (a), the sample of blood may be collected by any suitable means from the pregnant woman. The species free β-human chorionic gonadotrophin (free β-hCG) is distinct from the intact or total form of the molecule which is referred to simply as hCG or total hCG. The assay of the sample in step (b) for the levels of free β-human chorionic gonadotrophin (free β-hCG) and Inhibin A present in the sample may be carried out using standard protocols e.g. those based on ELISA (Enzyme-Linked ImmnoSorbent Assay) or RIA (RadioImmunoAssay) , or commercially available kits, e.g. free β-human chorionic gonadotrophin (free β-hCG) can be measured using the solid phase, two site fluoroimmunometric assay based on a direct sandwich technique as described by Stevenson *et al* (*Ann. Clin. Biochem.* **30** 99-100 (1993)) and Spencer *et al* (*Ann. Clin. Biochem.* **29** 506-518 (1992)). Inhibin A can be measured according to the solid phase sandwich ELISA assay described by Groome *et al* (*J. Immunol. Methods* **165** 167-176 (1993); *Clin. Endocrinol.*. **40** 717-723 (1994)). In particular embodiments of the present invention, the assay may also comprise an analysis of the levels of unconjugated oestriol (uE_3), which can be measured using the solid phase, time resolved fluoroimmunoassay described in US-A-4565790 and US-A-4808541. Additionally, since free β-hCG and total hCG are known to be highly correlated in pregnancy, total hCG may also be used as a screening marker for pre-eclampsia in a method according to the present invention as an alternative to free β-hCG. The intact hCG molecule (total

hCG) can be measured directly using exactly the same method as for the free β-subunit with AFP, i.e. sold phase, two-site fluoroimmunometric assay based on a direct sandwich technique. Preferably, the markers used are free β-hCG and Inhibin A measured after 20 weeks of pregnancy, and particularly at the end of the second trimester and the beginning of the third trimester.

In step (c), the determination of the risk of pre-eclampsia can be undertaken by comparing the levels of free β-human chorionic gonadotrophin (free β-hCG) and Inhibin A present in the sample with those in a control sample, or the median level in a group of control samples, i.e. samples from unaffected pregnancies, to provide a prediction of the probability of the onset of pre-eclampsia in the woman. The determination of risk may comprise deriving the likelihood ratio using a multivariate analysis based on distribution parameters from a set of reference data.

Calculation of risk from the measured marker levels is based on the observed relative frequency distribution of marker levels in (a) pre-eclamptic and (b) unaffected pregnancies. Any of the known statistical techniques may be used. Preferably the multivariate Gaussian model is used, which is appropriate where the observed distributions are reasonably Gaussian. Such multivariate Gaussian analysis is in itself known, for example from Wald NJ, Cuckle HS, Densem JW, et al (1988); Maternal serum screening for Down's syndrome in early pregnancy. BMJ 297, 883-887 and Royston P, Thompson SG (1992); Model-based screening by risk with application to Down's syndrome. Stat Med 11, 256-268.

In a preferred method, two Gaussian heights are calculated, (a) one for the pre-eclamptic distribution and (b) the other for the unaffected distribution. For this calculation, the necessary statistical parameters which specify the Gaussian distributions are the mean, the standard deviation and the correlations for the two distributions. The distributions are stored as reference data for use in analysis. The ratio of the two Gaussian heights

gives the likelihood ratio which is a measure of the increased risk of having a disorder, given a particular combination of test results, compared to the background risk, i.e. the risk in the absence of test results.

The estimation of risk consists of multiplying the likelihood ratio by the background risk for pre-eclampsia. The estimated risk is classified as screen-positive or screen negative based on a comparison with a predetermined risk cut-off. The value of the risk cut-off may be altered to vary the detection rate and false positive rate.

Methods in accordance with the present invention may further comprise the step (d) of re-expressing each measured screening marker level as a multiple of the median level of the respective screening marker in unaffected pregnancies of the same gestational age as the fetus of the pregnant woman. The screening marker levels may also be adjusted to allow for one or more factors selected from the group of maternal race, maternal weight, multiple birth and diabetic status.

According to a second aspect of the present invention there is provided an apparatus for determining whether a pregnant woman is at an increased risk of pre-eclampsia, the apparatus comprising:

(a) data input means for inputting a measurement of the serum levels of Inhibin A and free β-human chorionic gonadotrophin (free β-hCG) in a sample obtained from said pregnant woman; and

(b) calculation means for determining the risk of pre-eclampsia using the input levels of the serum markers Inhibin A and free β-human chorionic gonadotrophin (free β-hCG).

In certain embodiments of the invention, the calculation means may be arranged to

determine the risk of pre-eclampsia by deriving the likelihood ratio for pre-eclampsia using a multivariate analysis based on distribution parameters derived from a set of reference data. Preferably the multivariate analysis is a multivariate Gaussian analysis.

Apparatus in accordance with this aspect of the invention may further comprise (c) means for re-expressing the levels of each input screening marker Inhibin A and free β-human chorionic gonadotrophin (free β-hCG) as a multiple of the median level of the respective screening marker in unaffected pregnancies of the same gestational age as the fetus of the pregnant women and supplying the re-expressed screening marker levels to said calculation means.

According to a third aspect of the present invention there is provided a method of operating an apparatus as described in accordance with the second aspect to determine the risk of pre-eclampsia in a pregnant woman. The data input means may be used to enter items of data identified as the concentrations of serum markers Inhibin A and free β-human chorionic gonadotrophin (free β-hCG) in a sample obtained from a pregnant woman. The calculation means may be used to calculate the risk of pre-eclampsia using the input levels of the serum markers. The operation the different steps and preferred features are as described above. In another preferred embodiment of this aspect of the invention, the method comprises the steps described in Figures 4, 5, 6 and 7.

According to a fourth aspect of the invention there is provided a kit for predicting the onset of pre-eclampsia in a pregnant woman, comprising means for assaying a sample from the women for the levels of free β-human chorionic gonadotrophin (free β-hCG) and Inhibin A present in the sample.

Preferred features for the second and subsequent aspects of the invention are as for the first aspect *mutatis mutandis*.

The invention will now be further described by way of reference to the following Examples and Figures which are provided for the purposes of illustration only and are not to be construed as being limiting on the invention. Reference is made to a number of Figures in which:

FIGURE 1 shows a probability plot of the Inhibin levels in maternal serum in pre-eclampsia pregnancies (n=23) and unaffected pregnancies (n=96) collected before the onset of proteinuria. MoM = multiples of the normal median for unaffected pregnancies of the same gestational age.

FIGURE 2 shows a probability plot of the free β-hCG levels in maternal serum in pre-eclampsia pregnancies (n=22) and unaffected pregnancies (n=93). MoM = multiples of the normal median for unaffected pregnancies of the same gestational age.

FIGURE 3 shows a probability plot of the oestriol (uE_3) levels in maternal serum in pre-eclampsia pregnancies (n=13) and unaffected pregnancies (n=66). MoM = multiples of the normal median for unaffected pregnancies of the same gestational age.

FIGURE 4 shows a flowchart illustrating a screening method for pre-eclampsia that involves deriving a risk estimate from measurements made on biochemical samples collected during pregnancy.

FIGURE 5 shows a flowchart illustrating the procedure for calculating multiples of the median (MoM) for biochemical markers. LMP = last menstrual period.

FIGURE 6 shows a flowchart illustrating the procedure for adjusting MoM values to allow for various factors, other than gestational age, that may affect

biochemical marker levels.

FIGURE 7 shows a flowchart illustrating the procedure for selecting the appropriate parameters of the distributions of screening markers in affected and unaffected pregnancies. LMP = last menstrual period.

Example 1: Serum analysis

Serum analysis was carried out on serum collected between 1973 and 1975 from the John Radcliffe Maternity Hospital, Oxford, United Kingdom. Pre-eclampsia was defined as (i) a rise in systolic and diastolic pressure during pregnancy of 30 and 20mm of mercury respectively, compared with the level found at the first antenatal booking visit; (ii) proteinuria greater than 10mg % in a mid-stream urine sample; (iii) renal impairment as judged by the elevation of plasma uric acid levels of 6mg % or more. Nineteen women had blood samples taken after 12 weeks' gestation stored at -40°C. Nine women had one sample, seven had two samples, and three had three samples.

For each sample, three controls were identified selected at random from the patients attending the hospital who had provided a blood sample at the same gestational age in the same calendar quarter and were the same age. Neither cases nor controls were associated with Down's Syndrome or neural tube defects. Serum alphafetoprotein (AFP) and free β-human chorionic gonadotrophin (hCG) were measured using the Wallac-Delfia™ kit, unconjugated oestriol (uE_3) using the Ortho Clinical Diagnostics kit, and Inhibin A using the assay kit produced by Serotec. One sample was sufficient only to measure Inhibin A. For each serum marker, the logs of the medians for the controls were plotted by gestational age and a regression line fitted. The predicted marker values for each gestational age were estimated. All markers were expressed as multiples of their predicted median values for the controls, i.e. MoM's.

All analyses were also completed by using the marker values for each case expressed as

a multiple of the median value of its three controls. This removes the need to model the relationship of the markers with gestational age. The results did not differ significantly from those presented here.

The data were analysed using robust regression with the cluster option in STATA (Stata Corporation, Stata Statistical Software: Release 5.0, College Station, TX (1997)) to take account of repeat samples of some of the women. Table 1 shows the results for the four markers used, classified according to the onset of proteinuria. Inhibin A and free β-hCG values are raised in the pregnancies with pre-eclampsia and the level increases with decreasing time prior to proteinuria and is highest in women after the diagnosis of the disorder. Within three weeks of the onset of proteinuria, the mean Inhibin A value was 3.18 times the median for the controls (95% Confidence Interval - CI, 1.98-5.11), and the mean free β-hCG 3.43 (1.58-7.42). Even 10 weeks prior to the onset of proteinuria these two markers were elevated. The mean uE_3 was significantly reduced in the controls, within three weeks of the onset of proteinuria, MoM = 0.51 (95% CI, 0.42-0.62), but appears to rise again after the onset of proteinuria.

Table 2 shows the observed and expected (using the log Gaussian model) number of affected pregnancies above specified Inhibin A and free β-hCG levels. The correspondence is good. Based on multivariate Gaussian model using the parameters in Table 3 (based on results prior to the onset of proteinuria) in combination they yield an approximately 40% detection rate for a 5% false-positive rate as shown in Tables 5a and 5b.

Using the parameters in Table 4 (based on serum samples collected at 20 weeks gestation or later and before the onset of proteinuria) the detection rate for a 5% false-positive rate is 57% using free β-hCG and Inhibin A, or 67% using free β-hCG, Inhibin A and uE_3, as shown in Tables 5c and 5d.

The reduction in uE_3 needs to be investigated in further studies. These estimates are tentative because they are based on small numbers but provide an indication of the potential use of Down's Syndrome screening markers in the prediction of pre-eclampsia. It provides the opportunity to undertake randomised prevention trials in women at high risk of pre-eclampsia identified at the time of screening for Down's Syndrome, or later in pregnancy.

The results show that Inhibin A and free β-hCG are useful second trimester serum markers for pre-eclampsia. Each provided some independent predictive information because they were not totally correlated. Figures 1 and 2, and Table 2 demonstrate that both the Inhibin A and free β-hCG data fit log Gaussian distributions reasonably well.

The parameters in Tables 3 and 4 referred to above in Example 1 are calculated as follows. The mean $\log_{10}$ MoM in affected pregnancies is estimated from the $\log_{10}$ median value for each marker in affected pregnancies. The median MoM in unaffected pregnancies is 1.0 by definition, and so the $\log_{10}$ MoM value is 0. Standard deviations in affected and unaffected pregnancies are estimated from the slope of the regression lines fitted to the data in Figures 1, 2 and 3, between the 10^{th}-90^{th} centile range. Correlation coefficients between the markers in affected and unaffected pregnancies are estimated from the covariance between markers (after excluding values greater than 3.5 standard deviations from the mean), divided by the product of the standard deviations of the individual markers.

Example 2: Calculation of risk from measured marker levels

Most screening marker levels are known to vary with gestational age. To take account of this variation, each marker level may be expressed as a multiple of the median level (MoM) for unaffected pregnancies of the same gestational age. MoMs may be adjusted in a known way to take account of factors which are known to affect marker levels, such as maternal weight, ethnic group, diabetic status and the number of fetuses carried.

Calculation of risk from the measured marker levels is based on the observed relative frequency distribution of marker levels in (a) pre-eclamptic and (b) unaffected pregnancies. Any of the known statistical techniques may be used. Preferably the multivariate Gaussian model is used, which is appropriate where the observed distributions are reasonably Gaussian. Such multivariate Gaussian analysis is in itself known, for example from Wald NJ, Cuckle HS, Densem JW, et al (1988); Maternal serum screening for Down's syndrome in early pregnancy. BMJ 297, 883-887 and Royston P, Thompson SG (1992); Model-based screening by risk with application to Down's syndrome. Stat Med 11, 256-268. Thus no detailed discussion is necessary, but a summary is given as follows.

If a matrix representation is used, the height H of the Gaussian distribution for a given set of measured levels is given by the equation:

$$H = \frac{1}{\prod(\sigma)\cdot(2\pi)^{p/2}\cdot\det(\mathbf{R})^{1/2}}\exp\left(-\tfrac{1}{2}\cdot\mathbf{Z}^{T}\cdot\mathbf{R}^{-1}\cdot\mathbf{Z}\right)$$

where p is the number of markers, $\Pi(\sigma)$ is the product of the standard deviations for each distribution, **Z** is a matrix containing the measured level of each marker expressed in standard deviation units, namely ((measured level – mean) / standard deviation), and **R** is a matrix containing the correlations between the screening markers.

Two Gaussian heights are calculated, (a) one for the pre-eclamptic distribution and (b) the other for the unaffected distribution. For this calculation the necessary statistical parameters which specify the Gaussian distributions are the mean, standard deviation and correlations for the two distributions, as summarised in Table 4 (an improved set of parameters compared to the parameters given in Table 3) below for the preferred markers. The distribution parameters are stored as reference data for use in the analysis.

The ratio of the two Gaussian heights gives the likelihood ratio. The likelihood ratio is a measure of the increased risk of having a disorder, given a particular combination of test results, compared to the background risk (that is, the risk in the absence of the test results).

The likelihood ratio is multiplied by the known background risk, to calculate the improved estimate of risk. The estimated risk is classified as screen-positive or screen-negative based on a comparison with a predetermined risk cut-off. The value of the risk cut-off may be altered to vary the detection rate and false-positive rate.

Expected pre-eclampsia detection rates and false-positive rates using the present invention have been estimated using the method previously described in Wald NJ, Cuckle HS, Densem JW, et al (1988) referred to above. Tables 5a and 5b show the performance of screening for pre-eclampsia before the onset of proteinuria, using free β-hCG and Inhibin-A, in terms of the detection rate achieved at specified false-positive rates, and the false-positive rate required to achieve specified detection rates. Tables 5c and 5d show the performance of screening for pre-eclampsia at 20 weeks gestation or later, and before the onset of proteinuria, in the same terms.

Example 3: Computer algorithms for risk calculation

Figures 4 to 7 are flowcharts illustrating a specific method according to the present invention which is explained in detail below.

In the second trimester of pregnancy at around 14 to 22 weeks, a blood sample is drawn in step 1. Subsequently in step 2, the sample is assayed for the biochemical markers selected.

The processing of the measurements taken in step 2 is described below, and may be automated by implementing it in hardware or software.

Data input means are used to input the concentrations (levels) of the biochemical markers in step 3. In step 4, each marker level is re-expressed as a multiple of the median (MoM) level for unaffected pregnancies of the same gestational age and output as data item 5. Step 4 is illustrated in more detail in Figure 5. Stored data LMP 18 and scan 19 specific to respective methods of estimating gestational age are used to select an equation based on stored data which estimates the expected median concentrations for each marker at different gestational ages in step 20. Data LMP 18 is specific to estimation of gestational age based on the first day of the last menstrual period. Data scan 19 is specific to estimation of gestational age from an ultrasound measure of the fetus, usually a biparietal diameter (BPD) or crown-rump length (CRL) measurement. Based on an input in step 21 of the gestational age at the date of sample, for each marker in step 22 the expected median level in unaffected pregnancies of the same gestational age is calculated using the equation selected in step 20. In step 24 each marker level input in step 3 is divided by the expected median for that marker to output the MoM as data item 5.

Optionally the MoMs 5 for the biochemical markers may be adjusted in step 6 which is illustrated in detail in Figure 6. Based on an input of any one or more of maternal weight, ethnic group, diabetic status and the number of fetuses in steps 25 to 28, respectively, stored weight adjustment equations 29, ethnic group adjustments 30, diabetes correction factors 31, and multiple birth correction factors 32 are used in step 33 to adjust the MoMs 5. The adjusted MoMs are output as data item 7.

In step 8, a multivariate Gaussian analysis of the MoMs is performed. For use in this analysis, distribution parameters 10 are selected in step 9 which is described in more detail in Figure 7. For each marker the distribution parameters are stored as reference data 34 to 37 for different methods of estimating gestational age (LMP or scan) and based on whether or not the MoM has been adjusted for maternal weight. In step 38 the appropriate distribution parameters are selected and output as data item 10.

The multivariate Gaussian analysis 8 outputs a likelihood ratio as data item 11. In step 12 the likelihood ratio is multiplied by the stored background risk of pre-eclampsia 13 to output the estimated risk of pre-eclampsia as data item 14. The estimated risk 14 is compared with a predetermined cut-off in step 15 to produce a screen-positive result 16 when the risk is equal to or greater than the cut-off, or a screen-negative result 17 otherwise.

Table 1

Specified serum markers in pregnancies with pre-eclampsia according to timing of collection of serum samples relative to onset of proteinuria

Collection of serum sample relative to onset of proteinuria	Median gestation of onset of proteinuria	No. of women	No. of samples	Median gestation of serum samples	Geometric Mean (MoM) values (95% CI)			
					Inhibin A	AFP	Free β-hCG	uE_3
Over 11 weeks before	29.9	10	10	12.1	1.00 (0.75-1.32)	0.82 (0.61-1.11)	1.29 (1.95-1.76)	0.96 (0.48-1.94)
10-4 weeks before	29.4	6	6	21.5	1.26 (0.66-2.41)	1.13 (0.78-1.64)	2.09 (1.24-3.54)	0.87 (0.67-1.14)
3-0 weeks before*	28.9	6	7†	27.9	3.18 (1.98-5.11)	1.60 (0.58-4.42)	3.43 (1.58-7.42)	0.51 (0.42-0.62)
Up to 3 weeks after proteinuria*	29.9	5	9	32.3	6.66 (3.80-11.68)	1.36 (0.63-2.95)	3.98 (2.52-6.31)	0.93 (0.66-1.29)
Total*	29.9	19	32†	23.4	2.27 (1.52-3.38)	1.14 (0.83-1.57)	2.34 (1.66-3.28)	0.82 (0.60-1.12)
Total prior to onset proteinuria*	29.8	16	23†	21.1	1.49 (1.03-2.16)	1.07 (0.76-1.52)	1.92 (1.28-2.89)	0.78 (0.54-1.13)

MoM - multiples of the median

* - Standard errors adjusted for more than one sample from some women

† - One sample only had measurements of Inhibin

Table 2

Number and percentage of pregnancies with pre-eclampsia collected before onset of proteinuria and unaffected pregnancies according to Inhibin A and free β-hCG

MoM	Inhibin A				Free β-hCG			
	Affected no. (%) (n=23)	Modelled* %	Unaffected no. (%) (n=96)	Modelled* %	Affected no. (%) (n=22)	Modelled* %	Unaffected no. (%) (n=93)	Modelled* %
≥0.5	21 (91%)	93%	87 (91%)	90%	22 (100%)	97%	79 (85%)	84%
≥1.0	17 (74%)	70%	42 (44%)	50%	18 (82%)	82%	46 (49%)	50%
≥1.5	11 (48%)	50%	18 (19%)	22%	13 (59%)	63%	29 (31%)	28%
≥2.0	6 (26%)	35%	13 (14%)	9%	9 (41%)	48%	17 (18%)	16%
≥2.5	6 (26%)	25%	5 (5%)	4%	7 (32%)	36%	7 (8%)	9%
≥3.0	4 (17%)	18%	3 (3%)	2%	7 (32%)	27%	5 (5%)	5%

* - These percentages are estimated assuming both Inhibin A and free β-hCG have log normal distributions

Table 3

Distribution parameters of Inhibin A and free β-hCG in pregnancies with and without pre-eclampsia based on samples collected before onset of proteinuria (23 cases and 96 control samples)

		Log_{10} MoM	
		Inhibin A	Free β-hCG
Means	Unaffected	0	0
	Affected	0.164	0.284
Standard deviations	Unaffected	0.234	0.297
	Affected prior to onset of proteinuria	0.332	0.317
Correlation	Unaffected	0.198	
	Affected	0.899	

Table 4

Standard deviations, correlation coefficients, and means (log_{10} MoM) for selected screening markers in pregnancies with and without pre-eclampsia (affected and unaffected respectively); serum samples collected at 20 weeks gestation or later and prior to the onset of proteinuria (13 cases and 69 control samples)

	Inhibin A	Free β-hCG	uE_3
MEANS			
Free β-hCG	0	0	0
Inhibin-A	0.312	0.389	-0.164
STANDARD DEVIATIONS			
Unaffected	0.217	0.304	0.138
Affected	0.282	0.339	0.200
CORRELATION COEFFICIENTS			
Unaffected			
Free β-hCG	0.276	0.206	
uE3	0.037		
Affected			
Free β-hCG	0.893	-0.508	
uE3	-0.469		

Table 5a

Performance of pre-eclampsia screening using free β-hCG and Inhibin-A before onset of proteinuria: detection rates according to specified false-positive rates

False positive rate (%)	Detection rate (%).
3	36
4	40
5	43
6	45
7	48
8	50

Table 5b

Performance of pre-eclampsia screening using free β-hCG and Inhibin-A before onset of proteinuria: false-positive rates according to specified detection rates

Detection rate (%)	False-positive rate (%).
30	1.8
40	4.2
50	8.2
60	14

Table 5c

Performance of pre-eclampsia screening using free β-hCG, Inhibin A and uE3 measured in serum samples collected at 20 weeks gestation or later and before the onset of proteinuria: detection rates according to specified false-positive rates

False positive rate (%)	Detection rate (%)	
	Free β-hCG and Inhibin A	Free β-hCG, Inhibin A and uE_3
3	50	62
4	54	64
5	57	67
6	60	69
7	62	70
8	64	72

Table 5d

Performance of pre-eclampsia screening using free β-hCG, Inhibin A and uE3 measured in serum samples collected at 20 weeks gestation or later and before the onset of proteinuria: detection rates according to specified false-positive rates

Detection rate (%)	False-positive rate (%)	
	Free β-hCG and Inhibin A	Free β-hCG, Inhibin A and uE_3
30	0.4	0.04
40	1.2	0.2
50	2.9	0.8
60	6.0	2.5
70	12	6.9
80	22	16

CLAIMS

1. A method of predicting the risk of pre-eclampsia in a pregnant woman, the method comprising the steps of:

(a) obtaining a sample of blood from the woman;

(b) subsequently assaying the sample for the levels of free β-human chorionic gonadotrophin (free β-hCG) and Inhibin A present in the sample; and

(c) determining the risk of pre-eclampsia using the measure levels of free β-human chorionic gonadotrophin (free β-hCG) and Inhibin A present in the sample.

2. A method as claimed in claim 1 which further comprises assaying the sample for the levels of unconjugated oestriol (uE_3).

3. A method as claimed in claim 1 or claim 2 in which the method is carried out after 20 weeks of pregnancy,

4. A method as claimed in claim 3 in which the method is carried out at the end of the second trimester and the beginning of the third trimester.

5. A method as claimed in any one of claims 1 to 4 in which the determination of risk in step (c), is undertaken by comparing the levels of free β-human chorionic gonadotrophin (free β-hCG) and Inhibin A present in the sample with those in a control sample.

6. A method as claimed in claim 5, in which the determination of risk comprises deriving the likelihood ratio using a multivariate analysis based on distribution parameters from a set of reference data.

7. A method as claimed in claim 6, in which the multivariate analysis is a multivariate Gaussian analysis.

8. A method as claimed in 7, in which the estimation of risk consists of multiplying the likelihood ratio by the background risk for pre-eclampsia.

9. A method as claimed in any one of claims 1 to 8, the method further comprising a step (d) of re-expressing each measured screening marker level as a multiple of the median level of the respective screening marker in unaffected pregnancies of the same gestational age as the fetus of the pregnant woman.

10. A method as claimed in claim 9, in which the screening marker levels are adjusted to allow for one or more factors selected from the group of maternal race, maternal weight, multiple birth and diabetic status.

11. An apparatus for determining whether a pregnant woman is at an increased risk of pre-eclampsia, the apparatus comprising:

(a) data input means for inputting a measurement of the serum levels of Inhibin A and free β-human chorionic gonadotrophin (free β-hCG) in a sample obtained from said pregnant woman; and

(b) calculation means for determining the risk of pre-eclampsia using the input levels of the serum markers Inhibin A and free β-human

chorionic gonadotrophin (free β-hCG).

12. An apparatus as claimed in claim 11, in which the data input means (a) further comprises a data input means for inputting a measurement of the serum levels of unconjugated oestriol (uE_3) in a sample obtained from the pregnant woman.

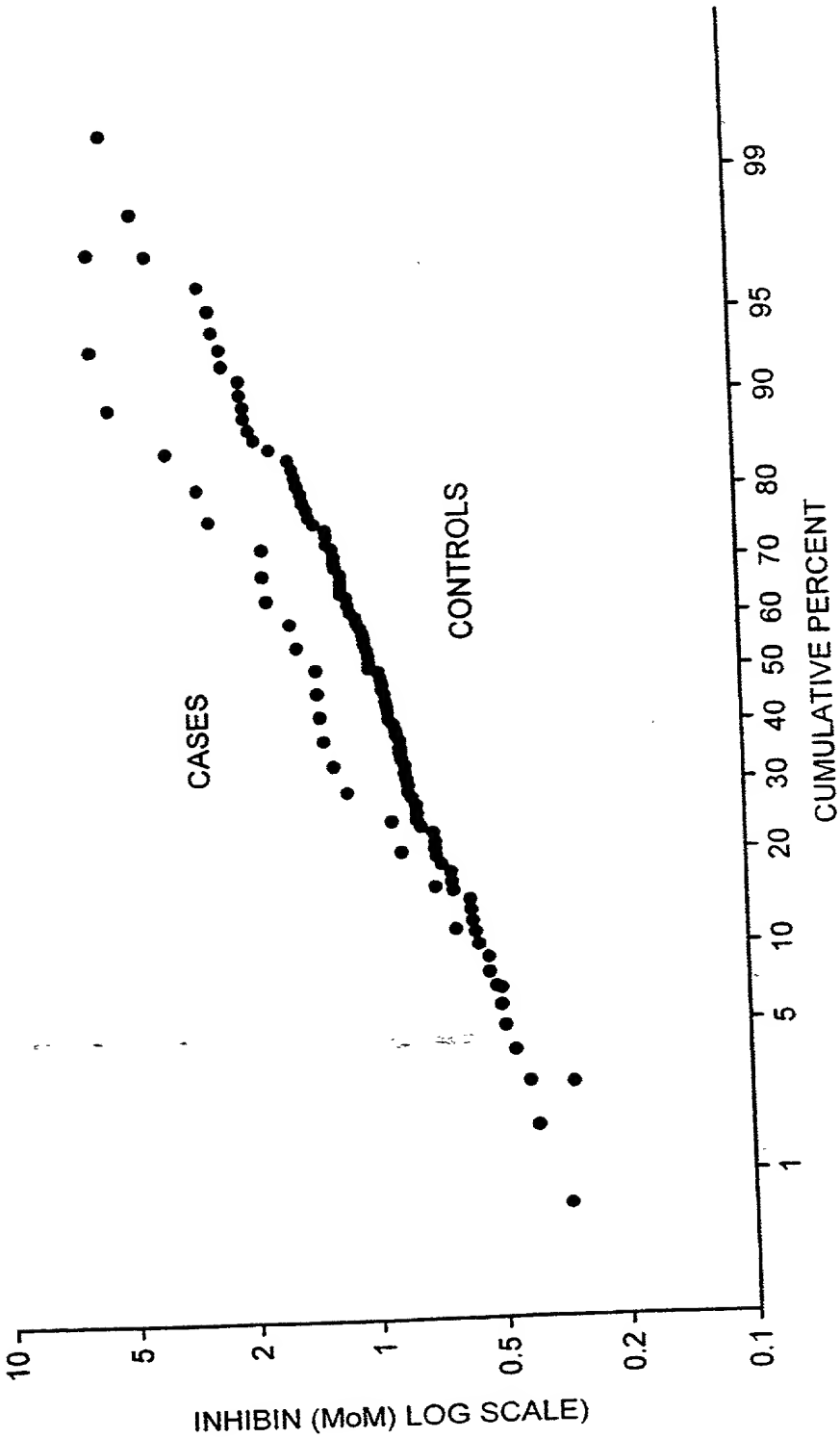
13. An apparatus as claimed in claim 11 or claim 12, in which the calculation means is arranged to determine the risk of pre-eclampsia by deriving the likelihood ratio for pre-eclampsia using a multivariate analysis based on distribution parameters derived from a set of reference data.

14. An apparatus as claimed in claim 13, in which the multivariate analysis is a multivariate Gaussian analysis.

15. An apparatus as claimed in any one of claims 11 to 14, in which the apparatus further comprises (c) means for re-expressing the levels of each input screening marker as a multiple of the median level of the respective screening marker in unaffected pregnancies of the same gestational age as the fetus of the pregnant women and supplying the re-expressed screening marker levels to said calculation means.

16. A kit for predicting the onset of pre-eclampsia in a pregnant woman, comprising means for assaying a sample from the women for the levels of free β-human chorionic gonadotrophin (free β-hCG) and Inhibin A present in the sample.

17. A kit as claimed in claim 16 which further comprises a means for assaying the sample for the levels of unconjugated oestriol (uE_3).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CUMULATIVE PERCENT
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0.2
0.1
INHIBIN (MoM) LOG SCALE)

FIG. 1

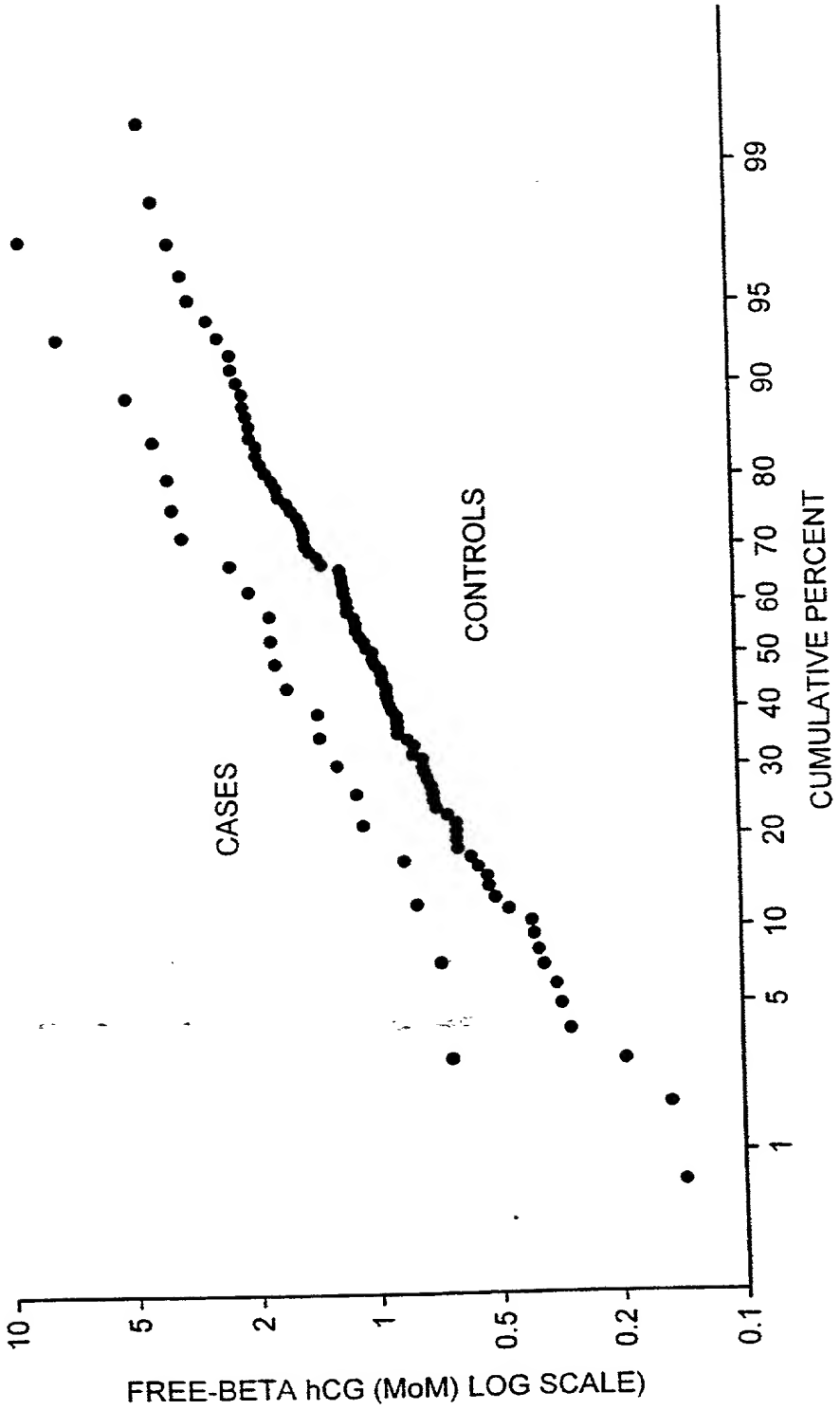
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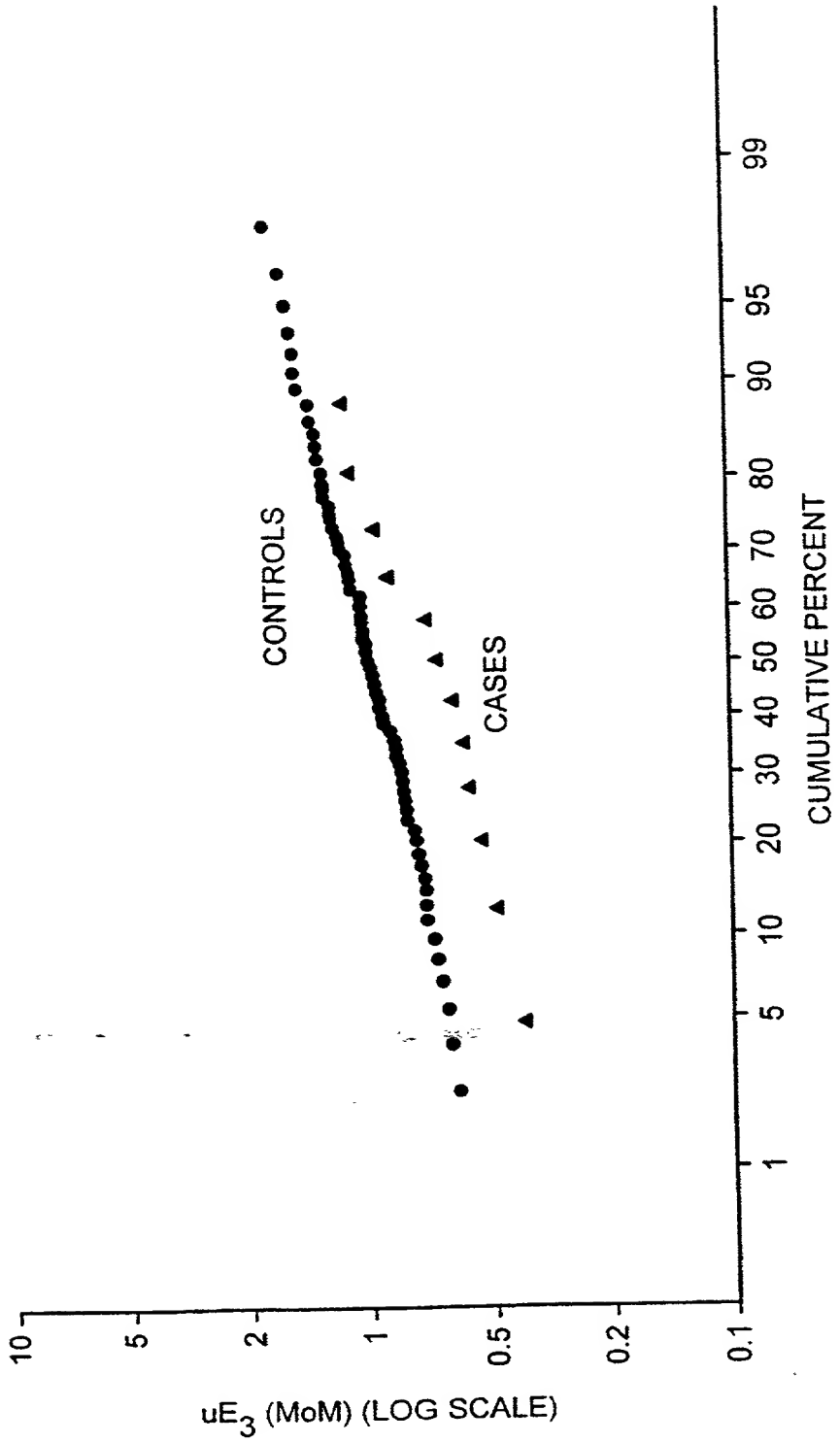
FIG. 2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uE3 (MoM) (LOG SCALE)

FIG. 3

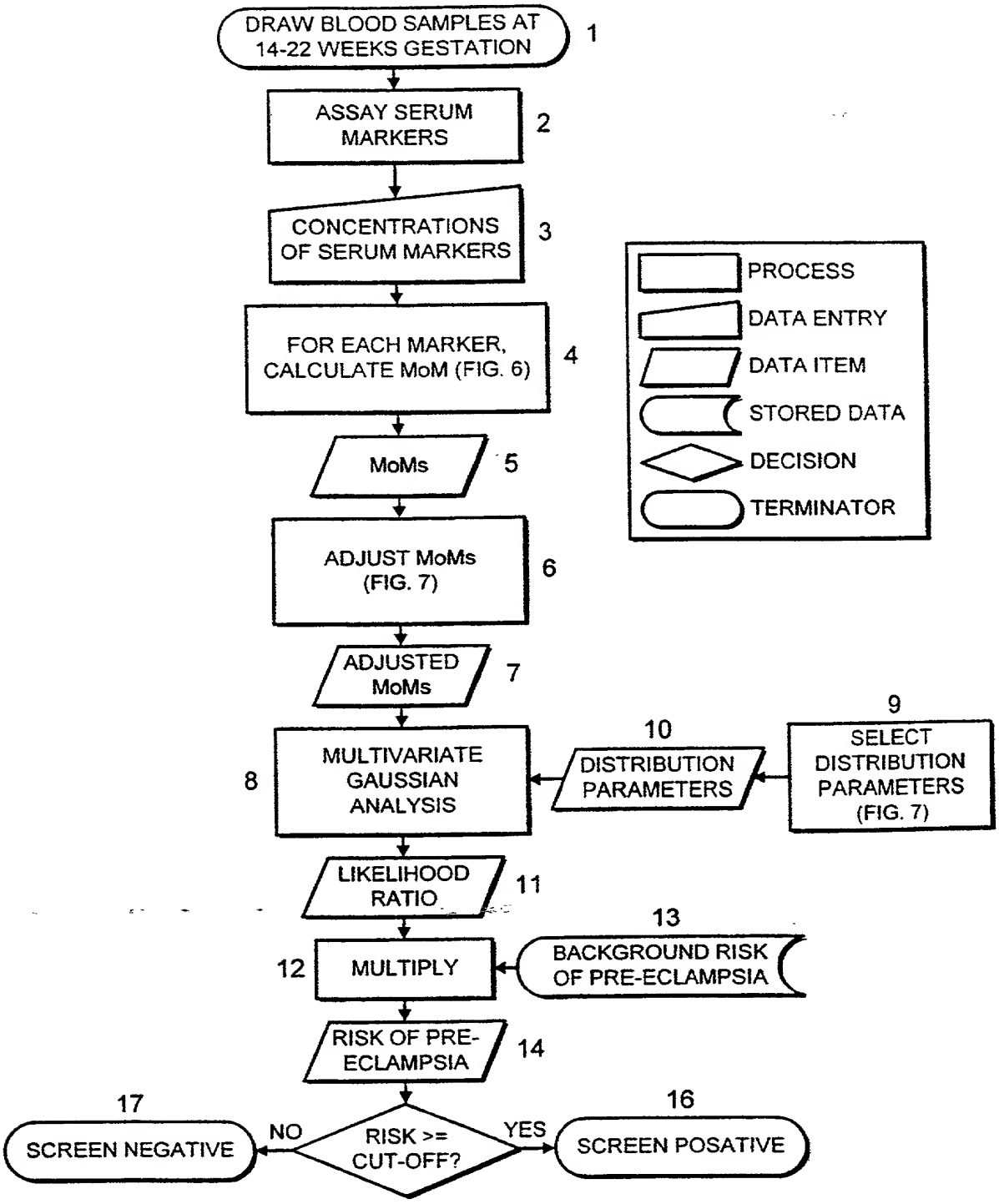
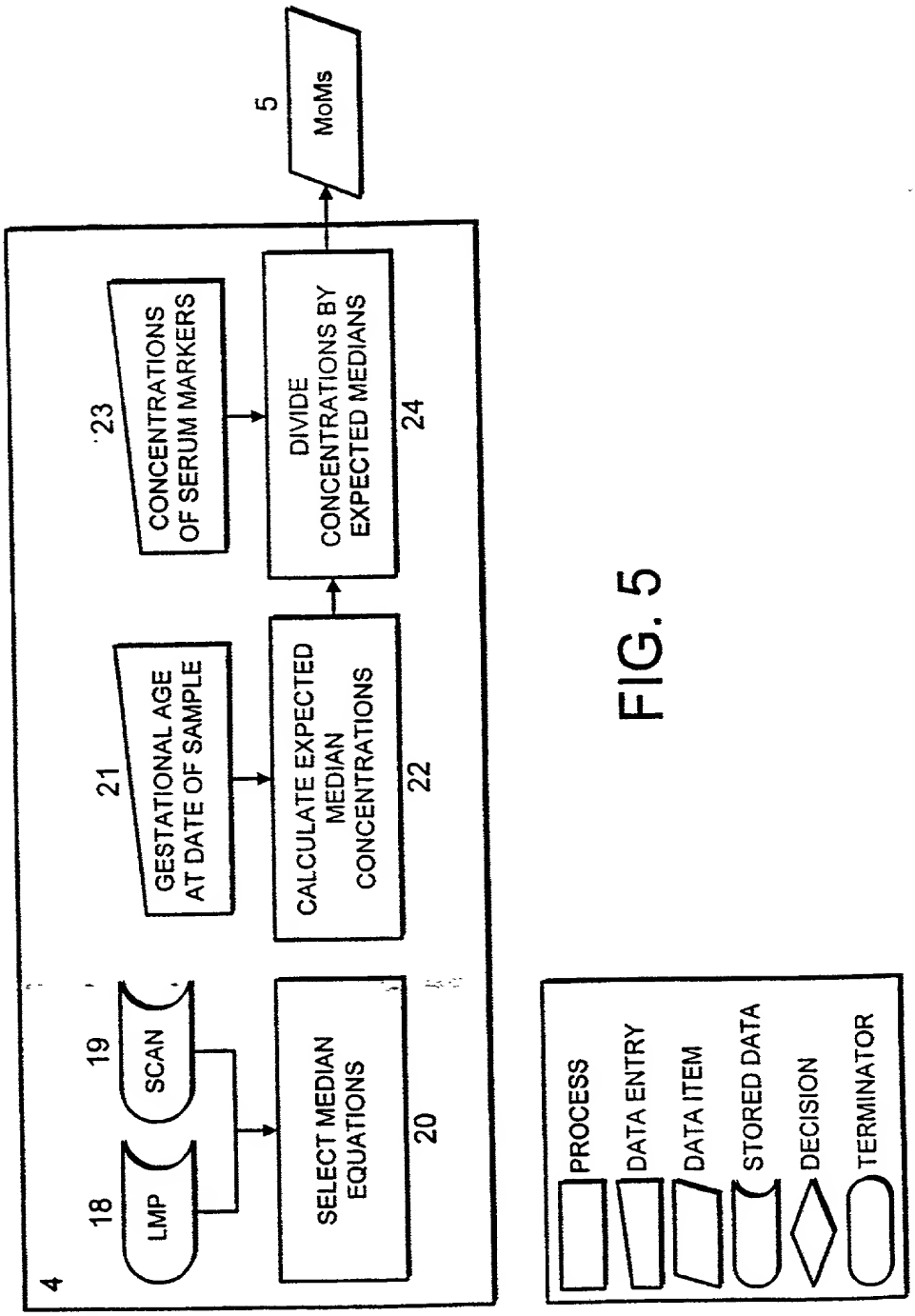

DRAW BLOOD SAMPLES AT 14-22 WEEKS GESTATION
1
ASSAY SERUM MARKERS
2
CONCENTRATIONS OF SERUM MARKERS
3
FOR EACH MARKER, CALCULATE MoM (FIG. 6)
4
MoMs
5
ADJUST MoMs (FIG. 7)
6
ADJUSTED MoMs
7
MULTIVARIATE GAUSSIAN ANALYSIS
8
DISTRIBUTION PARAMETERS
10
SELECT DISTRIBUTION PARAMETERS (FIG. 7)
9
LIKELIHOOD RATIO
11
MULTIPLY
12
BACKGROUND RISK OF PRE-ECLAMPSIA
13
RISK OF PRE-ECLAMPSIA
14
RISK >= CUT-OFF?
NO
YES
SCREEN NEGATIVE
17
SCREEN POSATIVE
16
PROCESS
DATA ENTRY
DATA ITEM
STORED DATA
DECISION
TERMINATOR

FIG. 4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MoMs
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CONCENTRATIONS OF SERUM MARKERS
DIVIDE CONCENTRATIONS BY EXPECTED MEDIANS
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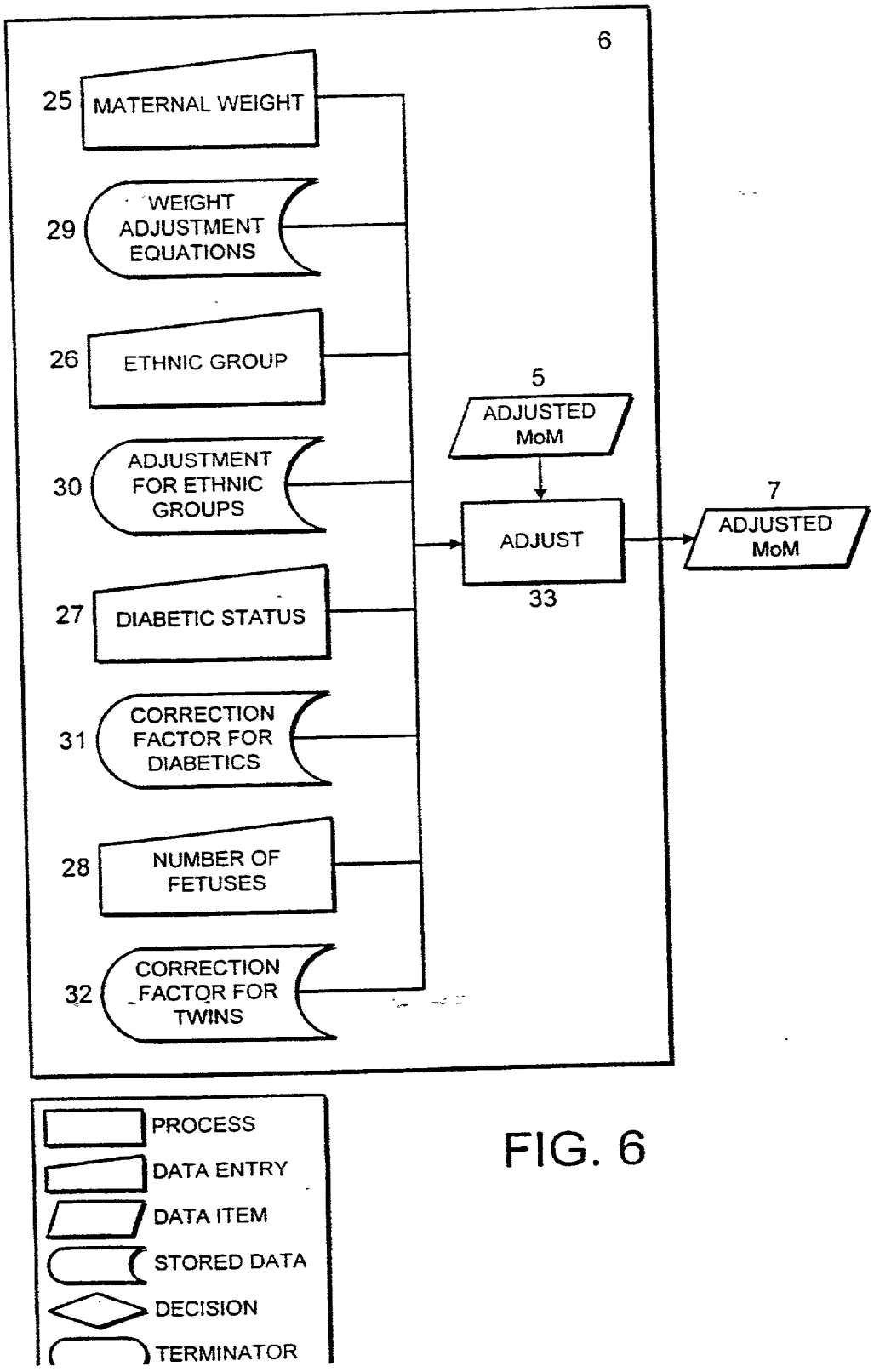
GESTATIONAL AGE AT DATE OF SAMPLE
CALCULATE EXPECTED MEDIAN CONCENTRATIONS
22
19
SCAN
18
LMP
SELECT MEDIAN EQUATIONS
20
4
PROCESS
DATA ENTRY
DATA ITEM
STORED DATA
DECISION
TERMINATOR

FIG. 5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30 ADJUSTMENT FOR ETHNIC GROUPS
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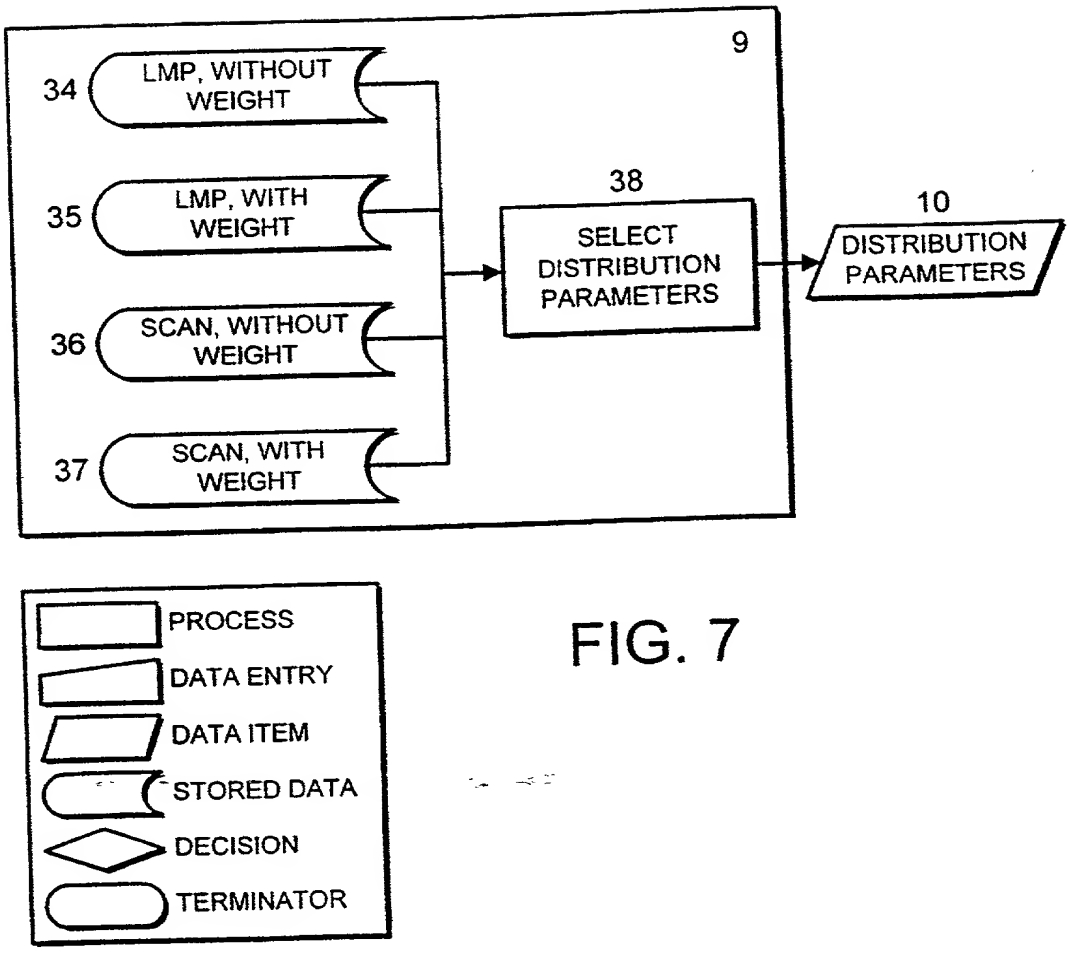
28 NUMBER OF FETUSES
32 CORRECTION FACTOR FOR TWINS
5 ADJUSTED MoM
ADJUST
33
7 ADJUSTED MoM
PROCESS
DATA ENTRY
DATA ITEM
STORED DATA
DECISION
TERMINATOR

FIG. 6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LMP, WITH WEIGHT
36
SCAN, WITHOUT WEIGHT
37
SCAN, WITH WEIGHT
38
SELECT DISTRIBUTION PARAMETERS
10
DISTRIBUTION PARAMETERS
PROCESS
DATA ENTRY
DATA ITEM
STORED DATA
DECISION
TERMINATOR

FIG. 7

Declaration for Patent Application

Docket Number: 0623.0950000/EKS/GLL

As a below named inventor, I hereby declare that:

My residence, mailing address and citizenship are as stated below next to my name.

I believe I am the original, first and joint inventor of the subject matter that is claimed and for which a patent is sought on the invention entitled **Predictive Test for Pre-Eclampsia**, the specification of which is attached hereto unless the following box is checked:

☒ was filed on December 7, 2000;
as United States Application Number 09/719,055 of PCT International Application Number PCT/GB99/01828;
Filed: June 9, 1999; and
was amended on _____ (if applicable).

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information that is material to patentability as defined in 37 C.F.R. § 1.56.

I hereby claim foreign priority benefits under 35 U.S.C. § 119(a)-(d) or § 365(b) of any foreign application(s) for patent or inventor's certificate, or § 365(a) of any PCT international application, which designated at least one country other than the United States listed below, and have also identified below any foreign application for patent or inventor's certificate, or PCT international application having a filing date before that of the application on which priority is claimed.

Prior Foreign Application(s)			Priority Claimed	
9812432.4	Great Britain	9 June 1998	☒ Yes	☐ No
(Application No.)	(Country)	(Day/Month/Year Filed)		
			☐ Yes	☐ No
(Application No.)	(Country)	(Day/Month/Year Filed)		

I hereby claim the benefit under 35 U.S.C. § 119(e) of any United States provisional application(s) listed below.

(Application No.)	(Filing Date)
(Application No.)	(Filing Date)

I hereby claim the benefit under 35 U.S.C. § 120 of any United States application(s), or under § 365(c) of any PCT international application designating the United States, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT international application in the manner provided by the first paragraph of 35 U.S.C. § 112, I acknowledge the duty to disclose information that is material to patentability as defined in 37 C.F.R. § 1.56 that became available between the filing date of the prior application and the national or PCT international filing date of this application.

(Application No.)	(Filing Date)	(Status - patented, pending, abandoned)
(Application No.)	(Filing Date)	(Status - patented, pending, abandoned)

Appl. No. 09/719,055
Docket No. 0623.0950000/EKS/GLL

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I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

1-00

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Signature of second inventor	Date
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Citizenship	United Kingdom
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Declaration for Patent Application

Docket Number: 0623.0950000/EKS/GLL

As a below named inventor, I hereby declare that:

My residence, mailing address and citizenship are as stated below next to my name.

I believe I am the original, first and joint inventor of the subject matter that is claimed and for which a patent is sought on the invention entitled **Predictive Test for Pre-Eclampsia**, the specification of which is attached hereto unless the following box is checked:

☒ was filed on December 7, 2000;
as United States Application Number 09/719,055 of PCT International Application Number PCT/GB99/01828;
Filed: June 9, 1999; and
was amended on _____ (if applicable).

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information that is material to patentability as defined in 37 C.F.R. § 1.56.

I hereby claim foreign priority benefits under 35 U.S.C. § 119(a)-(d) or § 365(b) of any foreign application(s) for patent or inventor's certificate, or § 365(a) of any PCT international application, which designated at least one country other than the United States listed below, and have also identified below any foreign application for patent or inventor's certificate, or PCT international application having a filing date before that of the application on which priority is claimed.

Prior Foreign Application(s)			Priority Claimed	
9812432.4	Great Britain	9 June 1998	☒ Yes	☐ No
(Application No.)	(Country)	(Day/Month/Year Filed)		
_____	_____	_____	☐ Yes	☐ No
(Application No.)	(Country)	(Day/Month/Year Filed)		

I hereby claim the benefit under 35 U.S.C. § 119(e) of any United States provisional application(s) listed below.

_____	_____
(Application No.)	(Filing Date)
_____	_____
(Application No.)	(Filing Date)

I hereby claim the benefit under 35 U.S.C. § 120 of any United States application(s), or under § 365(c) of any PCT international application designating the United States, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT international application in the manner provided by the first paragraph of 35 U.S.C. § 112, I acknowledge the duty to disclose information that is material to patentability as defined in 37 C.F.R. § 1.56 that became available between the filing date of the prior application and the national or PCT international filing date of this application.

_____	_____	_____
(Application No.)	(Filing Date)	(Status - patented, pending, abandoned)
_____	_____	_____
(Application No.)	(Filing Date)	(Status - patented, pending, abandoned)

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I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

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